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THE DETERMINATION OF AFM1 AND AFM2 IN THE CRUDE MILK BY HPLC IN THE PROVINCE OF DIYALA

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ABSTRACT

The residues of aflatoxin M1 and aflatoxin M2 may be present in the curde milk and milk products, which may cause health problems for the consumer, Crude milk collected from dairy farms in the Diyala Province, were isolated and examined for aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2). In this study 100 samples of milk were collected during the period between September to December 2017, the results revealed the presence of 12 isolate of fungal spp. And they are (Aspergillus flavus (17%), A. Terrace (7%), A. niger(48%), A. fumigates(6%), A. ochraceus (4%), Penicillum spp. (10%), Candidia spp.(31%), Rhodotorule spp.(23%), Fuserum(2%), Cladosporium (2%), Risobes spp. (4%) and Geotricum candidium (2%)). Aflatoxins are naturally occurring mycotoxins that are produced by Aspergillus flavus , This study also revealed the analysis of 15samples by HPLC to detect the concentration of AFM1 and AFM2 in milk samples which produced positive results of Aspergillus flavus where the results show the concentration of AFM1 vary from (0.26-0.00076) and for AFM2 vary from (0.1-0.00009) in the samples of the Crude milk.

KEYWORDS: Aflatoxin, AFM1, AFM2 & HPLC

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Original Article

INTRODUCTION

Mycotoxins have a multiplicity of adverse impact on the both humans and animals when ingested (Hampikyan et al., 2010). Aflatoxins are the toxic by-products produced mainly by two filamentous fungi; Aspergillus flavus and Aspergillus parasiticus (Baskaya et al., 2006). Other aflatoxin producing species include Aspergillus nomius, A. pseudotamarii, A. bombycis, A. toxicarius, A. parvisclerotigenus, A. ochraceoroseus, A. rambelliior the ascomycete genus Emericella by Emericellaastellata and E. venezuelensis (Ito et al., 2001;Frisvad et al., 2004; Frisvad et al., 2005; Reiter et al., 2009). Aflatoxins are polycyclic structures belonging to the furanocoumar in class of compounds, which are hepatotoxic, carcinogenic, and immunosuppressive fungal metabolites (Williams and others 2004) mainly produced by Aspergillus flavus and Aspergillus parasiticus (Prandini and others 2009). The most frequent aflatoxins are B1, B2, G1 and G2. Aflatoxin B1 (AFB1) is the most common toxic and has been reported as the most powerful natural carcinogen in the humans and animals (Hussain2008, Torkar & Vengust 2008). Aflatoxin M1 (AFM1) as a metabolite of AFB1 is created in the body of human and some mammalian animals. AFB1 is produced by some molds, including Aspergillus flavus, A. parasiticus, and rarely A. nomius occurring in some foodstuffs such as cereals, dried fruits, grains, nuts, milk, etc. (Atasever et al., 2014; Iqbal et al., 2015; Mason et al., 2015). Aflatoxin M1 (AFM1) or milk toxin is a hydroxylated metabolite of aflatoxin B1 and is secreted in the milk of dairy cattle after the consumption of feed

contaminated with aflatoxin B1 (Dashti et al., 2009, Iha et al., 2013). Aflatoxin M1 has been known to have cytotoxic, genotoxic, and carcinogenic effects (Fallah et al., 2010; Awad et al., 2012). It's the World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), U. S. Department of Agriculture (USDA), and U. S. Food and Drug Administration (FDA), among other organizations, categorize aflatoxin as a serious health risk and have established maximum levels for the occurrence of this toxin in food products. Food testing laboratories face the challenge of meeting regulatory requirements and implementing reliable and reproducible methods for identification of toxins and other hazards in order to ensure a safe food supply (Sani A et al., 2015). Aims of this study Isolation of A. flavus from crude animals milk from different area of diyala province, Extraction of aflatoxin and Detection of aflatoxin M1 and aflatoxin M2in milk by HPLC.

MATERIALS AND METHODS

Samples Collection

In this study 100 samples of curde milk were collected from (cow–sheep and goat) in Diyala province between September to December 2017. The milk samples were kept on ice during the transportation and cultured immediately upon arrival at the laboratory. These samples were cultured on Sabouraud Dextrose agar and Potato dextrose agar. Incubation was done at (25-27)°C for(3-5) days and checked daily for any growth.

Determination of AFM1 and AFM2

15 milk samples were analyzed for the presence of AFM1 and AFM2 using an immune affinity column for cleanup and HPLC. Extraction procedure according to (Sani A et al., 2015).

Milk samples were heated at 37 ±2°C and centrifuged for 15 minutes at 4000 rpm (2800 x g). After centrifugation, the upper fat layer was discarded and the sample was filtered with filter paper before being transferred to a 50 mL filtrate was taken in a syringe barrel which was attached with immune affinity columns (SPE). The test portion was passed at the flow rate of 2–3 mL/min. Two times of 10 ml purified water was passed through the column at a rate of 2–3 mL/min. eluted with 1.5 ml of acetonitrile. The 4 mL of collected extract was evaporated to dryness in a water bath at 50°C under a gentle stream of nitrogen The dry extract was then dissolved in 500 µL of mobile phase (water/acetonitrile, 30%, v/v), filtered through a syringe filter of modified PTFE membrane, and frozen until HPLC analysis.

HPLC was performed on fluorescence detector at wavelengths of 210,280 and 350 nm for excitation and emission, respectively. The column was (250 x 4.6 mm) 5 µmpartical size. The mobile phase consisted of acetic acid and the flow rate was 1 mL/min.

RESULTS

The results of this study revealed that twelve species isolated from 100 samples of crude milks collected from different locations of Diyala province. They are as follow Aspergillus flavus (17%), A. terreus (7%), A. niger (48%), A. fumigates (6%), A. ochraceus (4%), Penicillum spp. (10%), Candidia spp.(31%), Rhodotorule spp.(23%), Fuserum (2%), Cladosporium (2%), Risobes spp. (4%)and Geotricum candidium (2%).

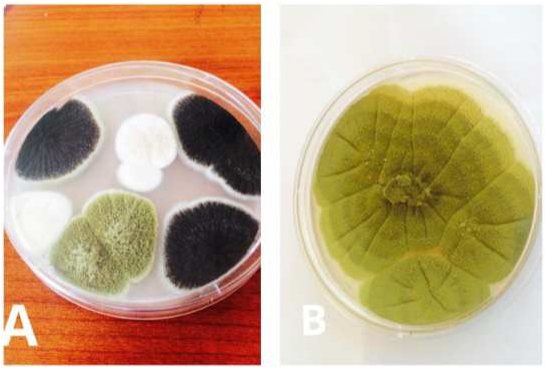

Figure 1: (A-Mix Culture Shows Different type of Aspergillus spp B-Pure Culture for Aspergillus Flavus)

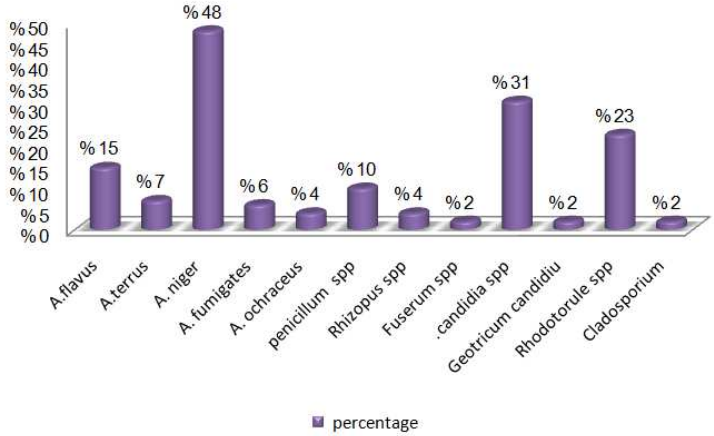

Figure 2: Percentage of Isolated Different Fungal Spp

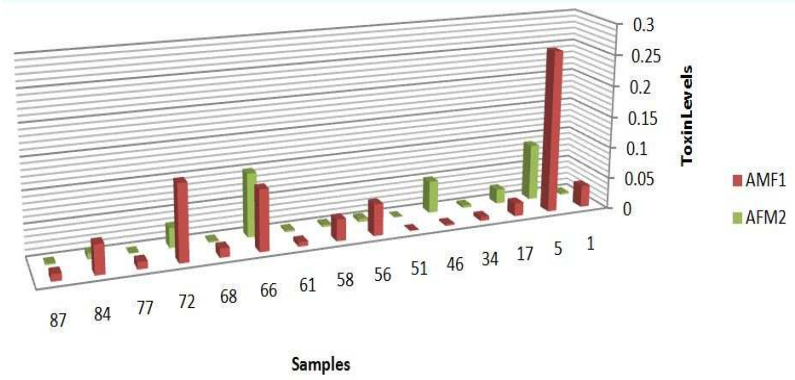

Figure 3: Comparison between AMF1 and AMF2 by Samples Size after Extraction by HPLC Acetonitrale Extraction

Minimum value AFM1 is (0.00076 and Maximum value is (0.26) and that the Minimum value AFM2 is (0.00009) and Maximum value is (0.1)).

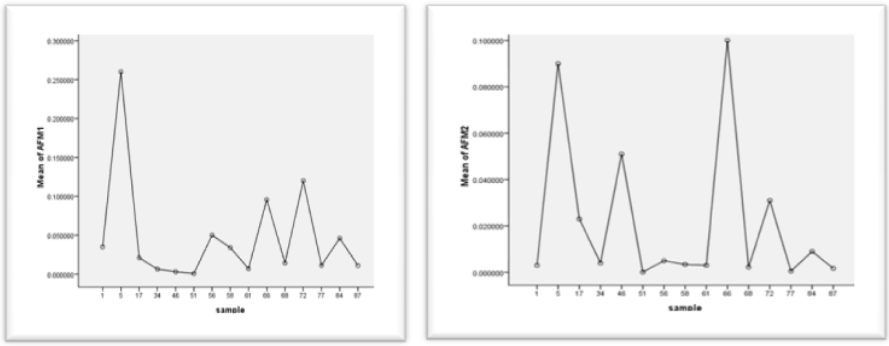

Figure 4: AFM1 and AFM2 Distribution by Samples Size

DISCUSSIONS

The results in the figure 2 showed the different types of fungi classified and this differs with reported by (GLADYS et al, 2017) fungi belonged to the genera Aspergillus3% (11/27), Geotrichum4.1% (15/27) and Fusarium0.3% (1/27), Candida 57.8% (211/365), Saccharomyces 6.6% (24/365), Cryptococcus 1.1% (4/365) and Rhodotorula27.1%.

In this study there occurs isolated yeast Candidiaspp and mold A. fumigatus_A. niger_A. terreus, penicillium spp_A. flavus that I agree with (Kubaysi,2008) in AL-anbar, the ratio A. flavu was identical to the ratio that recorded by (fadhel, 2008) in mousl 14.29%. The isolation of these types of fungi, may be attributed to many factors like Poor management, treatment with corticosteroid and antibiotic compound for long times.

The incidence and levels of AFM1 and A FM2 in samples are summarized in the figure 3 AFM1 and AFM2 was detected in 15 samples that give positive results to Aspergillus flavus during growth. AFM1 was detected at low level (<0.05ppb) in 11 (73.3%) of the samples, while 4 (26. 7%) sample had the highest value of >0.05pp b legal limit for the by European Union (EU).

The incidence of AFM1 and AFM2 observed in the present study was higher than the incidence of AFM1 and AFM2 reported by other authors [Galvano et al, 1998, kim et al, 2000, Rossi et al,. 2002]. The variations may be attributed to differences in region, season and specially analysis method.

In comparison with the recent data reporting the incidence of AFM1and AFM2 reported by others investigators (Galvano et al 2001, Garrido,. 2003)the results of this study is comparable with those presented in other countries, showing high incidence at low levels. According to Galvano et al. 1996, in recent years the incidence of AFM1 has been balanced on the one hand by the higher efficiency of analytical methods, and on the other hand by the setting of a stricter regulatory limit for aflatoxins in the feed and milk.

The controversies which were observed in the results of the previous studies could be attributed to some factors like seasonal variation, analysis methods, demographic characteristics, including clinical condition, diet type, etc.

In the above analysis of experimental data containing 100 samples for the purpose of calculating AFM1 and AFM2 concentration in milk collected in 15 sectors showed that a strong correlation was established by 72.2%. The size of the samples was not related to the concentration of these toxins. The variation of these samples was homogeneous indicating the homogeneity of the samples themselves.

CONCLUSIONS

This finding does not ignore the vital importance of exposure risk to this toxin in the consumers, especially children, based on the above results, the present situation is hopeful and might represent the possibility of altering standard limit of AFM1 concentration in milk in Iraq.

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